University of Groningen

Periconceptional health and lifestyle factors of both parents affect the risk of live-born children with orofacial clefts

Krapels, Ingrid P. C.; Zielhuis, Gerhard A.; Vroom, Fokaline; de Jong-van den Berg, Lolkje T. W.; Kuijpers-Jagtman, Anne-Marie; van der Molen, Aebele B. Mink; Steegers-Theunissen, Regine P. M.

Published in:

Birth Defects Research. Part A: Clinical and Molecular Teratology

DOI:

[10.1002/bdra.20285](https://doi.org/10.1002/bdra.20285)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2006

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Krapels, I. P. C., Zielhuis, G. A., Vroom, F., de Jong-van den Berg, L. T. W., Kuijpers-Jagtman, A-M., van der Molen, A. B. M., & Steegers-Theunissen, R. P. M. (2006). Periconceptional health and lifestyle factors of both parents affect the risk of live-born children with orofacial clefts. *Birth Defects Research. Part A: Clinical and Molecular Teratology*, 76(8), 613-620. <https://doi.org/10.1002/bdra.20285>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Periconceptional Health and Lifestyle Factors of Both Parents Affect the Risk of Live-Born Children with Orofacial Clefts

Ingrid P.C. Krapels,^{1,2} Gerhard A. Zielhuis,¹ Fokaline Vroom,³
Lolkje T.W. de Jong-van den Berg,³ Anne-Marie Kuijpers-Jagtman,²
Aebele B. Mink van der Molen,⁴ and Régine P.M. Steegers-Theunissen^{1,5–8*}
in collaboration with the Eurocran Gene-Environment Interaction Group

¹Department of Epidemiology and Biostatistics; Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

²Orthodontics and Oral Biology; Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

³Department of Social Pharmacy, Pharmacoepidemiology, and Pharmacotherapy, Groningen University Institute of Drug Exploration (GUIDE), Groningen, the Netherlands

⁴Department of Plastic and Reconstructive Surgery, Wilhelmina Children's Hospital, Utrecht, the Netherlands

⁵Departments of Obstetrics and Gynaecology, Division of Obstetrics and Prenatal Medicine, Erasmus MC University Medical Centre, Rotterdam, the Netherlands

⁶Department of Epidemiology and Biostatistics, Erasmus MC University Medical Centre, Rotterdam, the Netherlands

⁷Department of Pediatrics, Division of Paediatric Cardiology, Erasmus MC University Medical Centre, Rotterdam, Netherlands

⁸Department of Clinical Genetics, Erasmus MC University Medical Centre, Rotterdam, Netherlands

Received 30 January 2006; Revised 30 May 2006; Accepted 30 May 2006

BACKGROUND: Nonsyndromic cleft lip with or without cleft palate (CL/P) or cleft palate only (CPO) are orofacial clefts and have a multifactorial etiology. The identification of amendable parental risk factors may contribute to a reduced occurrence of these malformations in the future. **METHODS:** Standardized demographic and periconceptional exposure data from 284 parents of a child with CL/P, 66 parents of a child with a CPO and 222 parents of a child without congenital malformations were collected at ~24 months after the periconceptional period of the index child. Univariate and multivariate logistic regression analyses were used to estimate relative risks by odds ratios (ORs) and 95% confidence intervals (95% CIs). **RESULTS:** Univariate results suggest that low parental education, periconceptional maternal medication use and illnesses, paternal smoking, and first-trimester maternal common cold increased CL/P risk. Pregnancy planning and periconceptional folic acid supplementation, however, reduced CL/P risk by ~50% (OR, 0.5; 95% CI, 0.3–0.8) and 40% (OR, 0.6; 95% CI, 0.4–0.9), respectively. Mostly comparable results were obtained for CPO. Being a boy (OR, 2.0; 95% CI, 1.4–3.0), folic acid supplementation (OR, 0.6; 95% CI, 0.4–0.9), and low paternal education (OR, 1.6; 95% CI, 1.0–2.3) mainly determined CL/P in the multivariate analyses, compared to low paternal (OR, 4.5; 95% CI, 2.1–9.4) and maternal medication use (OR, 2.0; 95% CI, 1.0–4.0) for CPO. **CONCLUSIONS:** Preconceptional counseling for orofacial cleft risk assessment should pay attention to maternal medication use, periconceptional folic acid supplementation, and exposures of the father. These determinants can be amended, thereby modifying orofacial cleft risk. *Birth Defects Research (Part A) 76:613–620, 2006.* © 2006 Wiley-Liss, Inc.

Key words: cleft lip/palate; folic acid; lifestyle; maternal; parental; preconceptional; risk factors

Presented at the 51st Annual Meeting of the Society of Gynecologic Investigation, 22–26 March 2005, Houston, Texas, and the Annual meeting of the Dutch Society of Obstetrics and Gynecology, May 2005, Leiden, the Netherlands.

Grant sponsor: Fellowship of the Royal Netherlands Academy of Arts and Sciences (KNAW)(to R.S.T.); Grant sponsor: European Science Foundation (ESF), European Collaboration on Craniofacial Anomalies (EUROCRAN); Grant number: QLG1-CT-2000-01019.

Ingrid P. C. Krapel's current address: Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, the Netherlands.

*Correspondence to: Régine P.M. Steegers-Theunissen, MD, PhD, Associate Professor in Reproductive Epidemiology, Department of Obstetrics & Gynaecology, Erasmus MC, University Medical Center, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. E-mail: R.Steegers@erasmusmc.nl

Published online 5 September 2006 in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/bdra.20285

INTRODUCTION

Nonsyndromic orofacial clefts are congenital malformations with birth prevalence rates between 1 and 2 per 1000 live births. These birth defects are generally classified into cleft lip, alveolus with or without cleft palate (CL/P) and cleft palate only (CPO). The medical and psychosocial consequences of an orofacial cleft are rather severe and some children require multidisciplinary medical care up to adulthood. Therefore, there is a strong need to understand the causes in order to develop strategies to prevent these birth defects in the future.

The etiology of orofacial clefts is complex, and both genetic and environmental factors, such as health and lifestyles of both parents, are involved (Murray, 2002). The parental factors together with the genetic background differ considerably among populations and countries and may as such explain some of the ethnic and geographic variations in the epidemiology of these congenital malformations. In this context, the identification of both harmful and beneficial environmental factors may lead to intervention strategies to prevent orofacial clefts in newborns.

Most epidemiological studies in humans have focused on associations between orofacial clefts and maternal environmental factors, with inconclusive results. The maternal use of anticonvulsants (Murray, 2002; Reefhuis et al., 2002) and retinoids (Coberly et al., 1996; Murray, 2002), but also common environmental exposures, such as smoking (Lorente et al., 2000; Murray, 2002; Reefhuis et al., 2002), alcohol consumption (Lorente et al., 2000; Murray, 2002), maternal illnesses with fever (Zhang and Cai, 1993; Natsume et al., 2000; Murray, 2002; Reefhuis et al., 2002), and poor nutrition (Strauss, 1914; Krapels et al., 2004a, 2004b) have been associated with orofacial clefts. Preventive effects have been demonstrated for periconceptional food folate intake (van Rooij et al., 2004) and folic acid supplement use (van Rooij et al., 2003).

A harmful lifestyle of the father as a risk factor for orofacial clefts in the offspring has scarcely been evaluated (Zhang et al., 1992; Schnitzer et al., 1995; Shaw et al., 1996). It is plausible that exposures such as smoking, may exert a teratogenic effect through passive tobacco exposure of the mother or via direct exposure of spermatogenesis, thereby inducing genotoxic effects (DeMarini, 2004). Also, studies that focus on the periconceptional period of both parents-to-be are limited (Schnitzer et al., 1995; Shaw et al., 1996; van Rooij et al., 2001).

To reduce the birth prevalence rate of children with orofacial clefts in the future, we performed an explorative study to identify periconceptional health and lifestyle factors in both mothers- and fathers-to-be that affect the risk of having a child with nonsyndromic CL/P and CPO in the Dutch population. The generated information can be included in future preconceptional counseling of parents-to-be who are or are not at increased risk of having a child with an orofacial cleft.

MATERIALS AND METHODS

Between 1998 and 2003 a national case-control triad study (mother, father, child) was conducted at the Department of Epidemiology and Biostatistics of the Radboud University Nijmegen Medical Center in Nijmegen, and at the Department of Obstetrics and Gynecology of

the Erasmus MC, University Medical Centre in Rotterdam in the Netherlands. Details of the study design, case and control group have been described by us previously (van Rooij et al., 2001, 2003, 2004). In summary, children with a nonsyndromic orofacial cleft and both of their parents were approached via cleft lip and palate teams and the organization of parents of patients with clefts (VSOP/BOSK) in the Netherlands. Children with a Pierre Robin sequence were excluded. A total of 420 children with nonsyndromic orofacial clefts (343 CL/P and 77 CPO) and their parents participated and served as cases (compliance, 76%). In each center, the CL/P or CPO was diagnosed by a clinician according to a standard registration form (Luijsterburg and Vermeij-Keers, 1997). The control group ($n = 232$) consisted of parents and their child without a major congenital malformation recruited by either the case parent (61%) or nurseries and public health centers (39%), thereby covering the domain of the case group as much as possible. We excluded for the analysis non-Dutch and non-Caucasian triads (24 CL/P, 9 CPO, and 9 controls). Also excluded were families with either a family history of clefts, defined as the presence of ≥ 2 first-, second- or third-degree family members with either a CL/P or a CPO (35 CL/P, 2 CPO, and 1 control). This resulted in the participation of 350 cases (284 CL/P and 66 CPO) and 222 control families. The Central Committee for Medical Research in Human (The Hague) and the Medical Ethical Committees of all participating hospitals approved the study protocol, and written informed consent was obtained from all participating families.

We aimed to collect the data at a standardized time, ~24 months after the periconceptional period of the index pregnancy. The rationale for this study time is that most malformations and associated syndromes are identified during the first year of life, which is important for the proper selection of the controls. Moreover, it takes often >1 year to complete the diagnoses of the congenital malformation. With regard to recall and exposure assessment, this time was also chosen to facilitate recall, because it is in the same season as the periconceptional period of the index pregnancy. Both case and control parents completed the general questionnaire at home on demographics and periconceptional and first-trimester health and lifestyle exposures. All questionnaires were successfully checked in a standard way for completeness and consistency by the researcher. The type and dosages of folic acid and medication used during the periconceptional period were investigated during the interview and, in case of any doubt of either the participants or interviewer, we verified the information with the general practitioner or pharmacist. The maternal and paternal questionnaires covered the period of 3 months before until 3 months after conception and 3 months before until 2 weeks after conception, respectively. The period of 3 months before pregnancy for both parents was based on the duration of spermatogenesis (~70 days) and the fact that adequate folic acid status is reached after 3 months of folic acid supplementation.

As the first trimester is the most critical period for embryogenesis, we also asked the mothers about the occurrence of events during this period, such as the occurrence and the timing during pregnancy of severe nausea and/or vomiting, weight loss, and common colds (including influenza and ear, nose, and throat illnesses). Maternal nausea was characterized by duration, period, and severity. Severe nausea and/or vomiting

Table 1
Demographics of Mothers, Fathers, and Children and the Risk of Offspring with a Cleft Lip with or without Cleft Palate (CL/P) and Cleft Palate Only (CPO)

	CL/P <i>n</i> = 284	CPO <i>n</i> = 66	Controls <i>n</i> = 222	CL/P risk OR (95% CI)	CPO risk OR (95% CI)
Mothers					
Age, years, median (P5–P95) ^a	30.9 (25.4–39.1)	30.5 (25.8–36.5)	31.6 (26.0–37.3)	0.99 (0.95–1.04)	0.93 (0.86–1.01)
Low ^b education, <i>n</i> (%) yes	164 (57.8)	35 (53.0)	103 (46.4)	1.6 (1.1–2.3)	1.3 (0.8–2.3)
Primiparae, <i>n</i> (%) yes	118 (41.6)	36 (54.6)	115 (51.8)	0.7 (0.5–0.9)	1.1 (0.6–1.9)
Planned pregnancy, <i>n</i> (%) yes	242 (85.2)	59 (89.4)	204 (91.9)	0.5 (0.3–0.9)	0.7 (0.3–1.9)
Artificial fertilization, <i>n</i> (%) yes	23 (8.1)	3 (4.6)	15 (6.8)	1.2 (0.6–2.4)	NC
Spontaneous miscarriages ^c , <i>n</i> (%) yes	78 (27.5)	14 (21.2)	54 (24.3)	1.2 (0.8–1.8)	0.8 (0.4–1.6)
Use of oral contraceptives ^d , <i>n</i> (%) yes	66 (23.2)	16 (24.2)	52 (23.4)	1.0 (0.7–1.5)	1.1 (0.6–2.1)
Fathers					
Age, years, median (P5–P95) ^a	33.4 (27.1–41.3)	33.0 (27.7–39.4)	34.0 (28.5–43.2)	0.97 (0.94–1.01)	0.93 (0.87–0.99)
Low education ^b , <i>n</i> (%) yes	171 (60.2)	53 (80.3)	104 (46.9)	1.8 (1.2–2.5)	4.6 (2.4–8.9)
Children					
Boys, <i>n</i> (%)	191 (67.3)	25 (37.9)	114 (51.4)	2.0 (1.4–2.8)	0.6 (0.3–1.0)

^aAge at delivery of the index child. Data not available for 3 CL/P fathers and 1 control father.

^bLow: primary/ lower vocational/ intermediate secondary/ intermediate vocational education. Data not available for 3 CL/P fathers and 1 control father.

^cThe occurrence of spontaneous miscarriages before 16 weeks of pregnancy. Data not available for 1 control and 1 CL/P mother.

^dData not available for 1 CL/P, 2 CPO, and 1 control mother. OR, odds ratio; 95%CI, 95% confidence interval; NC, not computable as the cells contained numbers <5.

were defined as starting after the first week of pregnancy resulting in a changed or decreased food intake. Weight loss was defined as the loss of any weight in the first trimester of pregnancy, starting from the first day of pregnancy. The mothers were also asked whether and when they quit smoking or stopped consuming alcohol when pregnancy was confirmed. The maternal use of folic acid supplements was defined as the daily use from 4 weeks before until 8 weeks after conception and conforms to the recommendations of the Dutch government for all women who want to become pregnant. We also investigated the use of any folic acid supplement use during the periconceptional period. Data on maternal medication use comprised the dosage and frequency of intake and during which weeks of pregnancy the medication was taken. The main subgroups of medication were analgesics, antihistamines, antihypertensive drugs, ovulation-stimulating agents, corticosteroids, parasympathomimetics, antacids/antiemetics, antidepressants/anxiolytics/hypnotics, anticonvulsants, thyroid medication, sympathicomimetics (drugs with a sympathicomimetic action), prostaglandins, serotonin agonists, cytostatics, homeopathic drugs, anti-infectious drugs (antibiotics, antimycotics, antiviral, or antihelminthic drugs), and iron supplements. Other types of medication were categorized as "other." The parents could indicate illnesses, such as gastrointestinal disorders, diabetes mellitus, epilepsy, cancer, severe acne, allergies, malaria, psoriasis, and diseases of the thyroid, kidney, liver, intestine, stomach, pancreas, heart, thrombosis, and respiratory (asthma, bronchitis, or pneumonia), and urinary tract.

For statistical analyses all determinants were dichotomized except for age variables. Differences in age between case and control triads were tested by Wilcoxon's rank-sum test. The CL/P and CPO risks were estimated by odds ratios (ORs) with 95% confidence intervals (95% CIs). To assess dose-response effects of smoking, alcohol, or coffee consumption on orofacial cleft risk, we calculated ORs for the different strata. To avoid cells with

small numbers of observations, we sometimes pooled some of the subcategories. As the frequencies of cigarette and cigar/pipe smoking could not be combined, and most parents smoked cigarettes, we performed a dose-response analyses on this type of tobacco only.

When data on the timing of exposure were available, additional analyses were performed for which exposure was defined according to the critical period of the development of the cleft. For CL/P this is between the fifth and the 12th weeks after conception and between the seventh and the 12th weeks after conception for CPO.

To investigate the variables that mostly contributed to orofacial cleft risk, a multiple stepwise logistic regression analysis based on the outcomes of the univariate analysis was performed with a probability entry of the parameters with $P = 0.05$ and probability removal of the parameter of 0.10. An ROC curve was created in order to predict CL/P or CPO risk for the most contributing variables. For the statistical analyses, Statistical Analysis System (SAS) version 8.2 (SAS Institute, Cary, NC) was used.

RESULTS

The median (P5–P95) age of the children at the study time was 15.0 (10.8–24.0) years for the CL/P group, 15.6 (12.0–22.8) years for the CPO group, and 14.4 (8.4–22.8) years for the control group and were comparable between the groups. Table 1 indicates the demographics of the mothers and fathers associated with CL/P and CPO risk.

The periconceptional exposures of the mothers and fathers are outlined in Tables 2 and 3, respectively. So far, data are scarce on harmful paternal exposures and orofacial cleft risk in the offspring. Therefore, to gain more insight, we assessed a broad number of variables in an exploratory fashion. In an additional analysis, we restricted maternal smoking, alcohol consumption, medication use, and the occurrence of common cold to the critical periods of 5–12 weeks after conception for CL/P and

Table 2
Periconceptional Characteristics of the Mothers and the Risk of Offspring with a Cleft Lip
with or without Cleft Palate (CL/P) and Cleft Palate Only (CPO)

Periconceptional exposures ^a , n (%) yes	CL/P n = 284	CPO n = 66	Controls n = 222	CL/P risk OR (95%CI)	CPO risk OR (95%CI)
Lifestyle factors					
Smoking (cigarettes, cigars or pipe)	72 (25.4)	14 (21.2)	50 (22.5)	1.2 (0.8–1.8)	0.9 (0.5–1.8)
Cigarettes ^b					
None and <1/day	212 (74.7)	52 (78.8)	172 (77.5)	1.0 (Reference)	1.0 (Reference)
1–10/day	60 (21.1)	12 (18.2)	40 (18.0)	1.2 (0.8–1.9)	1.9 (0.5–2.0)
>10/day	11 (3.8)	2 (3.0)	10 (4.5)	0.9 (0.4–2.2)	NC
Alcohol ^c	112 (39.4)	31 (47.0)	108 (48.7)	0.7 (0.5–1.0)	0.9 (0.5–1.6)
None or <1/day	260 (91.6)	61 (92.4)	200 (90.1)	1.0 (Reference)	1.0 (Reference)
1 or more/day	18 (6.3)	3 (4.6)	17 (7.7)	0.8 (0.4–1.6)	NC
Coffee	205 (72.2)	47 (71.2)	165 (74.3)	0.9 (0.6–1.3)	0.9 (0.5–1.6)
None or less than 1/day	113 (39.8)	31 (46.9)	92 (41.4)	1.0 (Reference)	1.0 (Reference)
1–5/day	142 (50.1)	30 (45.4)	110 (49.6)	1.1 (0.7–1.5)	0.8 (0.5–1.4)
>5/day	29 (10.2)	5 (7.6)	20 (9.0)	1.2 (0.6–2.1)	NC
Folic acid supplements ^d	80 (28.2)	21 (31.8)	95 (42.8)	0.6 (0.4–0.8)	0.9 (0.5–1.6)
Any folic acid supplement use	173 (60.9)	45 (68.2)	151 (68.0)	0.7 (0.5–1.1)	1.0 (0.6–1.8)
Health factors					
Medication use ^e	105 (37.0)	26 (39.4)	58 (26.1)	1.7 (1.1–2.4)	1.9 (1.0–3.3)
Analgesics	19 (6.7)	5 (7.6)	13 (5.9)	1.2 (0.6–2.4)	NC
Ovulation inducing drugs	15 (5.3)	3 (4.6)	12 (5.4)	0.9 (0.4–1.9)	NC
Topical corticosteroids	10 (3.5)	3 (4.4)	5 (2.3)	1.4 (0.5–4.2)	NC
Sympathomimetics ^f	12 (4.2)	3 (4.6)	3 (1.4)	2.9 (0.8–10.3)	NC
Anti-infectious drugs ^g	24 (8.5)	7 (10.6)	12 (5.4)	1.6 (0.8–3.3)	NC
Iron supplements	10 (4.1)	3 (4.6)	9 (4.1)	1.0 (0.4–2.4)	NC
Illnesses ^h	136 (47.9)	29 (43.9)	77 (34.7)	1.7 (1.2–2.5)	1.5 (0.8–2.6)
Lungs ⁱ	17 (6.0)	4 (6.1)	9 (4.1)	1.6 (0.7–3.6)	NC
Urinary tract	7 (2.5)	1 (1.5)	7 (3.2)	0.7 (0.2–2.0)	NC
Allergy	39 (13.7)	9 (13.6)	25 (11.3)	1.3 (0.7–2.1)	1.2 (0.6–2.9)
First trimester					
Common cold ^j	61 (21.5)	15 (22.7)	32 (14.4)	1.7 (1.1–2.7)	1.7 (0.9–3.5)
Severe nausea and/or vomiting	37 (13.0)	11 (16.7)	38 (17.1)	0.8 (0.5–1.3)	0.9 (0.5–2.0)
Weight loss ^k	35 (12.3)	12 (18.2)	34 (15.3)	0.7 (0.4–1.2)	1.3 (0.6–2.6)
X-ray ^l	8 (2.8)	4 (6.1)	5 (2.3)	1.1 (0.4–3.5)	NC

^aDefined as exposure between 3 months before through 3 months after conception of the index child.

^bThe exact amount of cigarettes smoked was not available for 1 CL/P mother.

^cThe exact amount of alcoholic beverages was not available for 6 CL/P, 2 CPO, and 5 controls.

^dDaily use of supplements containing folic acid from 4 weeks before until 8 weeks after conception of the index child. Unknowns: 14 CPO, 8 controls, and 28 CL/P mothers; 99.6% of the CL/P mothers and all of the CPO mothers used a minimum of 400 µg of folic acid per day. One CL/P and 1 CPO mother used 5 mg of folic acid daily without a clear indication.

^eDefined as the use of any medication other than oral contraceptives, including iron supplements.

^fDefined as drugs with a sympathomimetic action, mainly consisting of Ventolin (7 CL/P, 1 CPO, 2 controls), followed by the decongestant Otrivin (3 CL/P, 3 CPO, 1 control).

^gDefined as antibiotics, antimycotics, antiviral drugs, antihelminthics.

^hDefined as any illnesses, common cold included.

ⁱDefined as asthma, bronchitis, or pneumonia. The largest subgroups of illnesses were asthmatic diseases (11 CL/P and 6 controls) and common cold (8 CL/P and 1 control).

^jIncluding influenza and ear, nose, and throat illnesses. Data not available for 4 CL/P and 1 control.

^kData not available for 1 CPO mother.

^lData not available for 1 CL/P mother. OR, odds ratio; 95% CI, 95% confidence interval; NC, not computable as the cells contained numbers <5.

of 7–12 weeks after conception for CPO. Most risk estimates remained the same or were reduced in strength. The association between common cold and CL/P, however, increased to OR, 2.2 (95% CI, 1.2–4.0).

It is conceivable that specific exposures could act synergistically on orofacial cleft risk if both parents are exposed. Therefore, we calculated the estimates for periconceptional smoking, alcohol consumption, and medication use of both parents with both nonexposed parents as a reference. Smoking by both parents tended to increase CL/P risk (OR, 1.4; 95% CI, 0.9–2.3) and CPO risk (OR, 1.4; 95% CI, 0.7–2.5), as did the use of alcohol by both

parents for CPO risk (OR, 1.9; 95% CI, 0.6–5.9). Both CL/P and CPO risk were markedly elevated when both parents used medication periconceptionally compared to nonusers (OR, 8.0; 95% CI, 1.8–35.5 and OR, 3.7; 95% CI, 0.5–27.2, respectively).

As expected, CL/P was more frequent among boys and CPO among girls. The exact mechanism for this finding is still unknown. In order to gain more insight in this gender-related clefting, we performed a stratified analysis for gender of the child per cleft type. Low paternal education and maternal medication use were important risk factors for CL/P as well as for CPO independent of the

Table 3
Periconceptional Exposures of the Fathers and the Risk of Offspring with Cleft Lip with or without Cleft Palate (CL/P) or Cleft Palate Only (CPO)

Periconceptional exposures ^a , <i>n</i> (%) yes	CL/P <i>n</i> = 284	CPO <i>n</i> = 66	Controls <i>n</i> = 222	CL/P risk OR (95% CI)	CPO risk OR (95% CI)
Lifestyle factors					
Smoking (cigarettes, cigars or pipe)	114 (40.1)	27 (40.9)	70 (31.6)	1.5 (1.0–2.1)	1.5 (0.9–2.6)
Cigarettes					
None or <1/day	179 (63.0)	41 (62.1)	157 (70.7)	1.0 (Reference)	1.0 (Reference)
1–10/day	40 (14.1)	8 (12.1)	28 (12.6)	1.3 (0.7–2.1)	1.0 (0.5–2.6)
>10/day	62 (21.8)	17 (25.8)	36 (16.2)	1.5 (1.0–2.4)	1.8 (0.9–3.5)
Alcohol	232 (81.7)	60 (90.9)	188 (84.7)	0.8 (0.5–1.4)	1.8 (0.7–4.4)
None or <1/day	152 (53.5)	36 (54.6)	123 (55.4)	1.0 (Reference)	1.0 (Reference)
1–3/day	105 (37.0)	26 (39.4)	77 (34.7)	1.1 (0.8–1.7)	1.2 (0.7–2.1)
>3/day	24 (8.5)	4 (6.1)	21 (9.5)	0.9 (0.5–1.7)	0.7 (0.2–2.0)
Coffee ^b	269 (94.7)	61 (92.4)	211 (95.1)	1.1 (0.5–2.5)	0.6 (0.2–1.8)
None or <1 cup/day	26 (9.2)	8 (12.1)	18 (8.1)	1.0 (Reference)	1.0 (Reference)
1–5 cups/day	138 (48.6)	26 (39.4)	96 (43.2)	1.0 (0.5–1.9)	0.6 (0.2–1.6)
6–10 cups/day	115 (40.6)	32 (48.5)	107 (48.2)	0.7 (0.4–1.4)	0.7 (0.3–1.7)
Health factors					
Medication use ^c	39 (14.7)	4 (6.1)	24 (10.8)	1.4 (0.8–2.3)	0.5 (0.2–0.6)
Analgesics	9 (3.2)	0	8 (3.6)	0.9 (0.3–2.3)	NC
Lungs	7 (2.5)	3 (3.0)	12 (5.4)	0.5 (0.2–1.2)	NC
Allergy	21 (7.4)	2 (3.0)	28 (12.6)	0.6 (0.3–1.0)	0.2 (0.1–0.9)

^aDefined as exposure between 3 months before through 2 weeks after conception of the index child. Data were missing for 3 CL/P and 1 control father.

^bExact amount of coffee consumed unknown for an additional 2 CL/P fathers.

^cDefined as the use of any medication, including iron supplements.

OR, odds ratio; 95% CI, 95% confidence interval; NC, not computable because the cells contained numbers <5.

gender of the child (data not shown). No other striking etiologic differences were found between the 2 sexes and cleft types and no conclusions could be drawn for the CPO group.

To determine the factors that contributed most to orofacial cleft risk, multiple stepwise logistic regression analyses was performed. The parameters that appeared to be a significant risk factor for orofacial cleft risk in the univariate analysis and those with a biological plausibility were included in the multivariate model. These variables comprised age, education, pregnancy planning, gender, parental smoking, alcohol consumption, medication use in general, maternal common cold between the fifth and 12th week after conception, and the daily use of folic acid supplements from 4 weeks before to 8 weeks after conception. The multivariate model revealed that the main determinants for CL/P risk were being a boy (OR, 2.0; 95% CI, 1.4–3.0), maternal folic acid supplement use (OR, 0.6; 95% CI, 0.4–0.9), and low educational status of the father (OR, 1.6; 95% CI, 1.0–2.3). The determinants in this model predicted CL/P with 64%. The same model for CPO revealed an area under the curve of 70%. Low paternal (OR, 4.5; 95% CI, 2.1–9.4) and maternal medication use (OR, 2.0; 95% CI, 1.0–4.0) were the most important factors for CPO.

In the analyses 61% of the controls were recruited from the population domain of the 10 cleft lip and palate teams in The Netherlands. However, due to the distance to the hospital and not having received ethical permission from some participating centers to recruit control families by the case families, we recruited 39% of the controls via nurseries and public health centers in the areas surrounding Nijmegen. Although the population in the Nether-

lands is very homogenous with regard to ethnicity, education, and exposures, in particular compared to the United States and United Kingdom, it is always possible that the associations found are due to residual confounding. Therefore, we performed additional analyses per cleft type for each of the 2 control groups separately. In these multivariate analyses, the most contributing factors comparing the case group to controls recruited via the cases (*n* = 135) were for the CL/P group pregnancy planning (OR, 0.5; 95% CI, 0.2–0.99) and the male gender of the child (OR, 1.9; 95% CI, 1.2–2.9), and for the CPO group low education of both parents (mother: OR, 0.4; 95% CI, 0.1–0.7; father: OR, 4.4; 95% CI, 1.9–10.3), maternal medication use (OR, 2.1; 95% CI, 1.0–4.4), and the occurrence of common colds between the fifth and 12th week after conception (OR, 3.0; 95% CI, 1.1–8.6). Comparing the case group to controls recruited via nurseries and public health centers revealed that for CL/P the periconceptional folic acid supplement use (OR, 0.4; 95% CI, 0.2–0.8), low maternal education (OR, 4.1; 95% CI, 2.2–7.5), the male gender of the child (OR, 2.8; 95% CI, 1.6–5.0), and maternal medication use (OR, 2.2; 95% CI, 1.1–4.4) were the main determining factors for CL/P; for CPO, low paternal education (OR, 10.1; 95% CI, 4.0–25.2), maternal folic acid supplement use (OR, 0.4; 95% CI, 0.2–0.9), and maternal medication use (OR, 3.0; 95% CI, 1.2–7.6) were the main determining factors. These results emphasize that socioeconomic status and educational level have a significant effect. However, adjustment of the univariate risk estimates for educational level did not alter our conclusions and low paternal education appeared to be an important contributing factor to both cleft types in the multivariate analyses.

DISCUSSION

This study provides a comprehensive picture of parental periconceptional and first-trimester lifestyle and health factors determining CL/P and CPO risk in the Dutch population. Our data substantiate the current knowledge on the importance of both genetic factors, for example, family history for clefts (Natsume et al., 2000) and the male gender (Shaw et al., 1991), and environmental risk factors, that is, low socioeconomic status reflected by low parental education (Clark et al., 2003), maternal medication use, and the use of folic acid supplements periconceptionally (van Rooij et al., 2003) in the pathogenesis of CL/P. Our results also emphasize the correct use of folic acid supplements in the recommended period, as any folic acid supplement use did not affect CL/P risk. According to our data, only low paternal education and maternal medication use, however, contributed to CPO risk.

Low paternal education is considered as a proxy for low socioeconomic status and lifestyle, for example, pregnancy planning and smoking, and seemed to be one of the most important risk factors for having a child with an orofacial cleft independent of the gender of the child or cleft type. This may indicate a common underlying mechanism. Our data are in line with that of others (Clark et al., 2003) but not all (Carmichael et al., 2003). Differences in the distribution of socioeconomic status in the various countries as well as the use of different definitions may explain the discrepancies in results. We speculate that a low socioeconomic status as a risk factor for orofacial clefts should be regarded in particular as a marker of parental health and lifestyle. Individuals with a low education tend to smoke more than those with a high education (Wardle and Steptoe, 2003), have a less healthy diet (Wardle and Steptoe, 2003), and are less familiar with proper folic acid supplement use in the periconceptional period (De Walle et al., 2002). These lifestyle factors, either alone or in combination with occupational activities and genetic background, play a role in the etiology of orofacial cleft. The trend data of the European Concerted Action on Congenital Anomalies and Twins (EUROCAT) registration reflecting the associations between congenital malformations and increasing socioeconomic status over time, however, are not in line with our findings (<http://www.rug.nl/umcg/faculteit/discipline-groepen/medischegenetica/eurocat>). This may be due to differences in the methodology of data collection by registries or in a case-control research setting. Moreover, an increasing socioeconomic status may also bring along an increased frequency of harmful exposures such as smoking and alcohol consumption. Nevertheless, our data emphasize the need for continued efforts to unravel the role of environmental risk factors in the etiology of nonsyndromic orofacial clefts.

It is generally accepted that CL/P and CPO are 2 different etiologic entities. Overall, our univariate data demonstrated comparable risk factors for both types of clefts. In the multivariate analyses it appeared that the male gender of the child and the use of periconceptional folic acid supplement use were the main determinants for CL/P risk, whereas for CPO risk, maternal medication use and low education of the father were the main risk

factors. Comparisons of the literature on folic acid (Itikala et al., 2001) and medication use and CPO are hampered by small numbers with inconclusive results. Based on our observations, we speculate that other pathways and genes not related or less sensitive to folate are involved in CPO. Similarly, alterations in pathways or genes involved in the detoxification of medications, such as the glutathione S-transferases, may explain the association between CPO and medication use. The timing of the use of the specific medications and folic acid supplement could have been implicated, especially as the development of the palate is only terminated at the 12th week after conception, whereas the Dutch recommendations for folic acid supplementation are until the 8th week. When we replaced the variable "medication use" in the multivariate analyses by "medication use during the 7th and 12th week of postconception," this variable was no longer a significant factor for CPO risk, underscoring that not the timing, but other medications or mechanisms are involved. The numbers, however, were too small to determine specific associations between medication use and CPO risk. We stress that the aforementioned results can be explained by the rather small numbers and therefore should be interpreted with caution. Most studies still focus on CL/P; nevertheless, we hope that these results will encourage further research on this congenital malformation, focused on the different cleft types and on CPO in particular.

In line with other studies, we found that CL/P was more prevalent among boys, whereas CPO prevailed among females (Shaw et al., 1991). The underlying mechanism is still unknown. Differences in maternal hormonal status (James, 2000), season of conception (Cagnacci et al., 2003), or increased paternal age (Rittler et al., 2004) have been suggested. These speculations could not be confirmed by our data, as paternal and child age at the study time, the use of oral contraceptives prior to conception, or the frequency of artificial reproduction were comparable between the case and control group. Other explanations may be gender-related differences in timing of embryonic development, the influence of genes or hormones beyond gonadal differentiation, or gender-specific mortality due to differences in ovum quality or placentation (Jongbloet, 2004; Rittler et al., 2004).

The issue of confounding due to recall bias always has to be discussed in case-control studies. Some variables, especially maternal common cold in the first trimester of pregnancy and parental medication use and illnesses in general, are prone to over- and underestimation, thereby affecting the risk estimates. Except for maternal alcohol consumption, however, the lifestyle data were in line with those reported in the Dutch population (Statistics Netherlands, 2003). Moreover, although recall bias can never be excluded, evidence is emerging that its role is considered to be rather small and merely nondifferential in case-control studies focused on congenital malformations (Swan et al., 1992; Infante-Rivard and Jacques, 2000).

Maternal illnesses and medication use in general in the periconceptional period appeared to increase orofacial cleft risk. Moreover, the use of medication in general by both parents in the periconceptional period increased both CL/P and CPO risk even more. Apart from common cold, the subgroups of medication use and illnesses were too small to draw firm conclusions. The increased

use of medication, however, was in line with the increased frequency of illnesses in the CL/P group (Feldman et al., 1989), a finding that strengthens the validity of the data. Data on the occurrence of fever during periods of common colds was not available. These weaknesses have to be considered when drawing conclusions from this data. Nevertheless, the health condition of the mother in particular appeared to interfere with palatogenesis of the child, as it increased orofacial cleft risk independent of gender and cleft type. We speculate that a wide range of teratogenic actions, such as genotoxicity of the medicines and microorganisms, alterations in maternal and fetal oxygen and nutritional supply, and the release of inflammatory factors, histamines, or other mediators may play a role in orofacial clefting. In this context, it would be interesting in future studies to investigate the role of the gene *IRF6* (interferon regulatory factor 6), which encodes for immunomodulatory properties and is described to be essential for normal palatogenesis (Zuccherro et al., 2004). The current knowledge on the teratogenic effects of medication is mainly based on animals. Studies in populations with high occurrences of specific illnesses and thus high exposure to specific medications may provide more insight into their interfering role in palatogenesis.

The strengths of our study compared to other studies is the short period between exposure and data collection as well as the standardization of the study time at which the questionnaires were filled out and verified. Moreover, the precise diagnosis of the type of cleft according to the Dutch Cleft Palate Association (NVSCA) registration and the rather large sample size of cases and controls distinguishes our study from others. The data derived from the separate analyses for the 2 control groups are based on small numbers but largely support the comparison of the total group, thereby substantiating its population-based character.

In conclusion, this study demonstrates that maternal periconceptional medication and folic acid supplement use seem to affect the occurrence of orofacial clefts in the Dutch Caucasian population. Nevertheless, it is plausible that other, socioeconomically-related factors are also involved. This information deserves emphasis in the preconception counseling of parents-to-be in order to reduce the occurrence of these congenital malformations in future generations.

ACKNOWLEDGMENTS

We thank the participating cleft lip and palate teams, the parents and patient organization for clefts (VSOP/BOSK) and their coordinators (VSOP/BOSK, Mr. B van Beek; Rijnstate Hospital Arnhem, Dr. W. Brussel; Free University Medical Centre, Amsterdam and Erasmus University Medical Centre, Rotterdam, Prof. Dr. B. Prahlandersen; University Medical Centre Groningen, Prof. Dr. S.M. Goorhuis-Brouwer; Radboud University Nijmegen Medical Centre, Prof. Dr. A-M Kuijpers-Jagtman; Wilhelmina Children's Hospital, Utrecht; Dr. A.B. Mink van der Molen; Medical Centre, Leeuwarden, Dr. J.J. van der Biezen; St. Elisabeth Hospital, Tilburg, Dr. J.G. Dagers; Sophia Hospital, Zwolle, Dr. P. Hout; and Leiden University Medical Centre, Dr. F Ras). We thank the nurseries and the public health centers for their participation in the recruitment of control triads. We thank Mrs.

M. van der Doelen and Dr C. van Oostrom, chief nurse officer and head of the outpatient pediatric clinic, Radboud University Nijmegen Medical Centre, respectively, for their assistance, Dr. C. Vermeij-Keers for the opportunity to use the standardized registration form of the Dutch Association for cleft palate and craniofacial anomalies, Mrs. A. Pellegrino and Mrs. L. Lemmens for data entry, and Mr. W. Lemmens for data management. Finally, we acknowledge the collaboration within the Eurocran Gene-Environment Interaction Group.

REFERENCES

- Cagnacci A, Renzi A, Arangino S, et al. 2003. The male disadvantage and seasonal rhythm of sex ratio at the time of conception. *Hum Reprod* 18:885-887.
- Carmichael SL, Nelson V, Shaw GM, et al. 2003. Socio-economic status and risk of conotruncal heart defects and orofacial clefts. *Paediatr Perinat Epidemiol* 17:264-271.
- Clark JD, Mossey PA, Sharp L, Little J. 2003. Socioeconomic status and orofacial clefts in Scotland, 1989 to 1998. *Cleft Palate Craniofac J* 40: 481-485.
- Coberly S, Lammer E, Alashari M. 1996. Retinoic acid embryopathy: case report and review of literature. *Pediatr Pathol Lab Med* 16:823-836.
- DeMarini DM. 2004. Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. *Mutat Res* 567:447-474.
- De Walle HE, Cornel MC, de Jong-van den Berg LTW. 2002. Three years after the Dutch folic acid campaign: growing socioeconomic differences. *Prev Med* 35:65-69.
- EUROCAT. European Concerted Action on Congenital Anomalies and Twins Homepage. Time trend of cleft palate. Time trend of cleft lip with or without cleft palate. Available at: <http://www.rug.nl/umcg/faculteit/disciplinegroepen/medischegenetica/eurocat/pdf/CleftPalate.pdf> and <http://www.rug.nl/umcg/faculteit/disciplinegroepen/medischegenetica/eurocat/pdf/CleftLipWithOrWithoutCleftPalate.pdf>. Accessed: July 14, 2006.
- Feldman Y, Koren G, Mattice K, et al. 1989. Determinants of recall and recall bias in studying drug and chemical exposure in pregnancy. *Teratology* 40:37-45.
- Infante-Rivard C, Jacques L. 2000. Empirical study of parental recall bias. *Am J Epidemiol* 152:480-486.
- Itikala PR, Watkins ML, Mulinare J, et al. 2001. Maternal multivitamin use and orofacial clefts in offspring. *Teratology* 63:79-86.
- James WH. 2000. Are orofacial clefts a consequence of maternal hormonal imbalance? Evidence from the sex ratios in sibs of probands. *Teratology* 62:342-345.
- Jongbloet PH. 2004. The ovopathy concept for explaining the secondary sex ratio. *Hum Reprod* 19:1036-1038.
- Krapels IPC, van Rooij IALM, Ocké MC, et al. 2004a. Maternal dietary B vitamin intake, other than folate, and the association with orofacial cleft in the offspring. *Eur J Nutr* 43:7-14.
- Krapels IPC, van Rooij IALM, Ocké MC, et al. 2004b. Maternal nutritional status and the risk for orofacial cleft offspring in humans. *J Nutr* 134: 3106-3113.
- Lorente C, Cordier S, Goujard J, et al. 2000. Tobacco and alcohol use during pregnancy and risk of oral clefts. Occupational Exposure and Congenital Malformation Working Group. *Am J Public Health* 90: 415-419.
- Luijsterburg AMJ, Vermeij-Keers C. 1999. NVSCA-registration cleft lip/alveolus and cleft palate. Annual Report. Department of Plastic Surgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands. 14 p.
- Murray JC. 2002. Gene-environment causes of cleft lip and/or palate. *Clin Genet* 61:248-256.
- Natsume N, Kawai T, Ogi N, Yoshida W. 2000. Maternal risk factors in cleft lip and palate: case control study. *Br J Oral Maxillofac Surg* 38:23-25.
- Reefhuis J, de Jong-van den Berg LT, Cornel MC. 2002. The use of birth defect registries for etiological research: a review. *Community Genet* 5:13-32.
- Rittler M, Lopez-Camelo J, Castilla EE. 2004. Sex ratio and associated risk factors for 50 congenital anomaly types: clues for causal heterogeneity. *Birth Defects Res A Clin Mol Teratol* 70:13-19.
- Schnitzer PG, Olshan AF, Erickson JD. 1995. Paternal occupation and risk of birth defects in offspring occupation. *Epidemiology* 6:577-583.
- Shaw GM, Croen LA, Curry CJ. 1991. Isolated oral cleft malformations: associations with maternal and infant characteristics in a California population. *Teratology* 43:225-258.

- Shaw GM, Wasserman CR, Lammer EJ. 1996. Orofacial clefts, parental cigarette smoking, and transforming growth factor- α gene variants. *Am J Med Genet* 528:551–561.
- Statistics Netherlands. 2003. General Social Survey (POLS), 2003 (Dutch) <http://statline.cbs.nl>.
- Strauss OA. 1914. Predisposing causes a of cleft palate and harelip. *Transactions of the Sixth International Dental Congress*, London. pp. 470–471.
- Swan SH, Shaw GM, Schulman J. 1992. Reporting and selection bias in case-control studies of congenital malformations. *Epidemiology* 3: 356–363.
- van Rooij IALM, Wegerif MJM, Roelofs HMJ, Peters WH, et al. 2001. Smoking, polymorphisms in biotransformation enzymes, and nonsyndromic oral clefting: a gene-environment interaction. *Epidemiology* 12:502–507.
- van Rooij IALM, Groenen PM, van Drongelen M, et al. 2002. Orofacial clefts and spina bifida: N-acetyltransferase phenotype, maternal smoking, and medication use. *Teratology* 66:260–266.
- van Rooij IALM, Vermeij-Keers C, Kluijtmans LAJ, et al. 2003. Does the interaction between maternal folate and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol* 157:583–591.
- van Rooij IALM, Ocké MC, Straatman H, et al. 2004. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. *Prev Med* 39:689–694.
- Wardle J, Steptoe A. 2003. Socioeconomic differences in attitudes and beliefs about healthy lifestyles. *J Epidemiol Community Health* 57: 440–443.
- Zhang J, Savitz DA, Schwingl PJ, Cai WW. 1992. A case-control study of paternal smoking and birth defects. *Int J Epidemiol* 21:273–278.
- Zhang J, Cai WW. 1993. Association of common cold in the first trimester of pregnancy with birth defects. *Pediatrics* 92:559–563.
- Zuccherro TM, Cooper ME, Maher BS, et al. 2004. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N Engl J Med* 351:769–780.